Association of vectors and environmental conditions during the emergence of Peruvian horse sickness orbivirus and Yunnan orbivirus in northern Peru

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Received 22 April 2015; Accepted 9 July 2015

ABSTRACT: Since 1983, cases of diseased donkeys and horses with symptoms similar to those produced by alphaviruses were identified in two departments in northern Peru; however serological testing ruled out the presence of those viruses and attempts to isolate an agent were also unproductive. In 1997, also in northern Peru, two new orbiviruses were discovered, each recognized as a causative agent of neurological diseases in livestock and domestic animals and, at the same time, mosquitoes were found to be infected with these viruses. Peruvian horse sickness virus (PHSV) was isolated from pools of culicid mosquitoes, *Aedes serratus* and *Psorophora ferox,* and Yunnan virus (YUOV) was isolated from *Aedes scapularis* in the subtropical jungle (upper jungle) located on the slope between the east side of the Andes and the Amazonian basin in the Department of San Martín. Both viruses later were recovered from mosquitoes collected above the slope between the west side of the Andes and the coast (Department of Piura) in humid subtropical areas associated with the Piura River basin. In this region, PHSV was isolated from *Anopheles albimanus* and YUOV was isolated from *Ae. scapularis*. We discuss the ecology of vector mosquitoes during the outbreaks in the areas where these mosquitoes were found. ***Journal of Vector Ecology* 40 (2): 355-363.**

Keyword Index: Peru, orbivirus, Peruvian horse sickness orbivirus, Yunnan orbivirus, mosquitoes, *Aedes serratus, Aedes scapularis, Psorophora ferox*, ecology.

INTRODUCTION

During a Venezuelan equine encephalitis virus (VEEV) surveillance program in Peru in 1983, attempts to isolate a virus from equids with clinically-observed encephalitis were unsuccessful. During subsequent years, similar but sporadic cases were detected but no virus was isolated. In 1997, an epizootic outbreak involving domestic animals (equids, bovids, ovids, and canids) occurred and, using electron microscopy, a reovirus was detected. Later, two new orbiviruses were identified from domestic animals and mosquitoes (Attoui et al. 2009). The orbiviruses (family *Reoviridae*, genus *Orbivirus*) Peruvian horse sickness virus (PHSV) and Yunnan orbivirus (YUOV) were isolated from whole blood and brain tissues of sick animals and from chronically infected animals, as well as from mosquitoes. The affected departments were San Martín, adjacent to the northeastern side of the Andes, and Piura, located on the northwest side (Figure 1). In San Martín, most of the affected animals were equids but in Piura bovids were primarily affected.

Phylogenetically, PHSV, the orbivirus identified in vertebrates and in mosquitoes, belongs to the same serotype as Elsey virus, which had been isolated from horses with neurological disease in northern Australia (Melville 2004). PHSV and Elsey viruses share a high degree of sequence identity, particularly in the gene encoding the outer capsid protein VP3 (99%). Alternatively, Middle Point orbivirus, a virus isolated from an apparently healthy sentinel bovid in northern Australia, and YUOV, although closely related to each other and distinct from PHSV, are distinct serotypes (Cowled et al. 2007). Yunnan orbivirus was originally isolated from *Culex tritaeniorhynchus* mosquitoes in China (Attoui et al. 2005).

Peru encompasses diverse ecosystems, with 84 of the recognized 114 life zones of the world and 28 of the 34 recognized climates. In these ecosystems, climate events occur non-dramatically but may cause climatic alterations, which benefits or inhibits human development or biological development in general. The aim of this study was to describe the environs in which we found these viruses and vectors in Peru by comparing mosquito abundance with weather data and human activities during years in locations where there were arbovirus infections.

Figure 1. Map of Peru. The principal locations mentioned in the text, Department of Piura and Department of San Martín.

MATERIALS AND METHODS

Locations

San Martín. The Department of San Martín occupies an area of 51,253 km^2 in northeastern Peru. The Huallaga River basin constitutes the principal watershed of this region and is the focal point of a hydrographic system of 126 rivers. The region is hilly and comprises four morphological zones: (1) a western zone bordering the eastern slope of the Andes, (2) a central zone of wide valleys, with terraces formed by the Huallaga River and its tributaries, (3) a southeast zone, with elevations not exceeding 3,000 m, and (4) a northeast zone of lowlands.

In 1997, an orbiviral disease outbreak appeared in the central zone, associated with the Huallaga River and one of its tributaries, the Mayo River. This area has a predominantly humid tropical climate, ecologically known as "upper jungle," and an altitude between 223-860 m above sea level, a mean temperature between 22° and 35° C, and an annual rainfall of 900 -1,500 mm. Precipitation levels have two distinct temporal peaks in the basin, the first generally occurs between March and April and the second, usually in October, associated with discharges of tributary rivers. The course of the Huallaga River in these broad valleys is associated with small lagoons and broader flood areas (IIAP 2009).

The region's main economic activity is agriculture. The basin of the Huallaga River has one of the largest areas of natural pastures (more than 36,400 ha) in Peru. Extensive cattle ranching occupies 60% of this area; raising other livestock is a secondary activity.

Over the past two decades, the rural human population in northeastern Peru has grown rapidly, giving rise to the occupation and exploitation of unprotected forests. In spite of governmental efforts, many regions have suffered indiscriminate logging of large areas, resulting in erosion of soil, landslides, floods, and a decreased seasonal supply of water (Gentry and López-Parodi 1980). Designation of the high forest as a protected zone has failed to save the forest and its biodiversity. One of the areas most affected by the orbivirus epizootic was the upper Mayo River basin, which corresponds to the Rioja and Moyobamba provinces. The human population of the upper Mayo River area, about 140,000, is involved in agriculture, the economic and productive base of the region. The most important products in the area are corn, rice in the lower (irrigated) areas, and coffee in the upper part. The approximately 60,000 residents of the rural areas of the upper Mayo River tend cattle, sheep, pigs, horses, and poultry. In 1997, the cattle population was 150,000 and there were 20,000 sheep, 100,000 pigs, and 8,000 horses. Cattle and sheep spend their days grazing on natural pastures close to the homes of their owners, the pigs feed in the poultry yards, and the horses accompany agricultural operations for transport of firewood, crops, and other products.

Mosquitoes were captured for virus isolation during the outbreak in March, 1997. Collections were made in the secondary forest where horses are allowed to rest in the provinces of Mariscal Cáceres (7° 10′ 57.134″ S, 76° 44′ 27.151″ W) and Moyobamba (6° 6′ 28.3278″ S, 76° 55′ 28.4406″ W and 6° 4′ 0.5412″ S, 77° 5′ 18.1494″ W). For two consecutive days, 10-11 March, we collected mosquitoes manually from 10:00 to 12:00 and from 16:00 to 19:00, using resting horses as bait and suction tubing by mouth. Mosquitoes collected were placed in plastic cups, covered, and stored in a refrigerator until the next day. They were then identified to species and stored in plastic tubes, each containing an average of 25 mosquitoes. Tubes were then stored in liquid nitrogen for transportation to the laboratory in Lima.

Piura. The Department of Piura, with a total area of 35,892 km^2, is located in northwestern Peru, and incorporates a long coast, its weather influenced by the Humboldt current and the periodic warm or cool currents of El Niño/La Niña-Southern Oscillation (ENSO). The Piura River basin comprises an area of approximately 12,200 km^2, from its headwaters at 3,600 m above sea level to its mouth at the Pacific Ocean. Ecologically, Piura encompasses the high arid plains of the Andes, with perpetual snow, equatorial dry forest, tropical valleys formed by rivers originating in the basin of the Amazonian high forest, and subtropical deserts along the southern Pacific coast (Rodriguez 2005), all of which interact with the warm current of the El Niño and the cold Humboldt current of the Pacific Ocean.

In the Department of Piura, the average annual humidity is 66%. On the coast, at 100 m and 500 m above sea level, the low side of the Piura River basin, rainfall ranges from 10-200 mm, and at 500 m to 1,500 m above sea level, the high side of the Piura River basin, rainfall ranges from 200-800 mm.

At the beginning of 1997, there occurred an anomaly of temperature, reaching record high levels during the epizootic in the months of July and August. The El Niño occurred from December, 1997 to May, 1998 and had a greater impact on the economy and the ecology of Piura than that which occurred in 1982-1983 (Woodman 1998).

The human population of the Piura River basin is more than 900,000. Almost 40% of them are active in agriculture, livestock, hunting, and forestry. Floods occur annually in the rainy period, which is concentrated between January and April. The distribution of crops in the basin of the Piura River is related to water availability and weather conditions. Crops raised on the high side of the Piura River basin vary widely.

The areas affected by the epizootic of YUOV in cattle and donkeys in this department were in districts from the contiguous provinces of Ayabaca (Suyo) and Morropón (Yamango), the high side of the Piura River basin, including the border with neighboring Ecuador. During the YUOV epizootic disease season, the area where the largest number of cattle deaths occurred was in the province of Morropón, which has a human population of about 170,000, 56,000 cattle, 35,000 sheep, and 35,000 swine. The Yamango district in eastern Morropón province was the most highly impacted by the epizootic. Cases were recorded in an area located at an altitude of 1,175 m above sea level where the climate is mainly warm. The months of January-March are relatively cold. Maximum temperature is 25° C and minimum 16° C. Agricultural activity includes some cattle, equids (mainly donkeys), poultry, and a few sheep. A characteristic of the agricultural activity in Yamango is that during the rainy season cattle are moved to areas at higher altitude where there are lush native grasses. When rains occur the animals are kept in dry quarters called "greenhouses." After the rains, the animals are moved to pens near human abodes where they are fed with dry grass from May to December.

The main economic activity in Suyo involves livestock, with about 7,000 cattle, 17,000 goats, 15,000 poultry, and 1,000 sheep. In this area farmers mainly grow rice and corn, leaving vast uncultivated areas for access by grazing animals. There frequently is trade for Ecuadorian cattle without sanitary controls. In addition, vast expanses of forest here are used by locals for fuel, producing an altered environment. In the affected areas, the rainy season coincides with the highest temperature. In 1997 the temperature reached 27.2° C in February. YUOV cases were reported from July to August (dry season) similar to reports from the Department of San Martín but for a shorter period of time (Figure 2). At that time we alerted all of Peru and asked that cases of livestock with neurological symptoms be reported to local health authorities.

Mosquitoes were collected as they were feeding on donkeys in the District of Suyo in Ayabaca Province (4° 30' 36.389" S, 80° 0' 16.900" W), 14 March 2008, from 17:00-19:00. In the District of Yamango (5° 10' 47.914" S, 79° 45' 10.882" W), we attempted to collect mosquitoes on 14 June 2010 during the morning from animals in greenhouses but

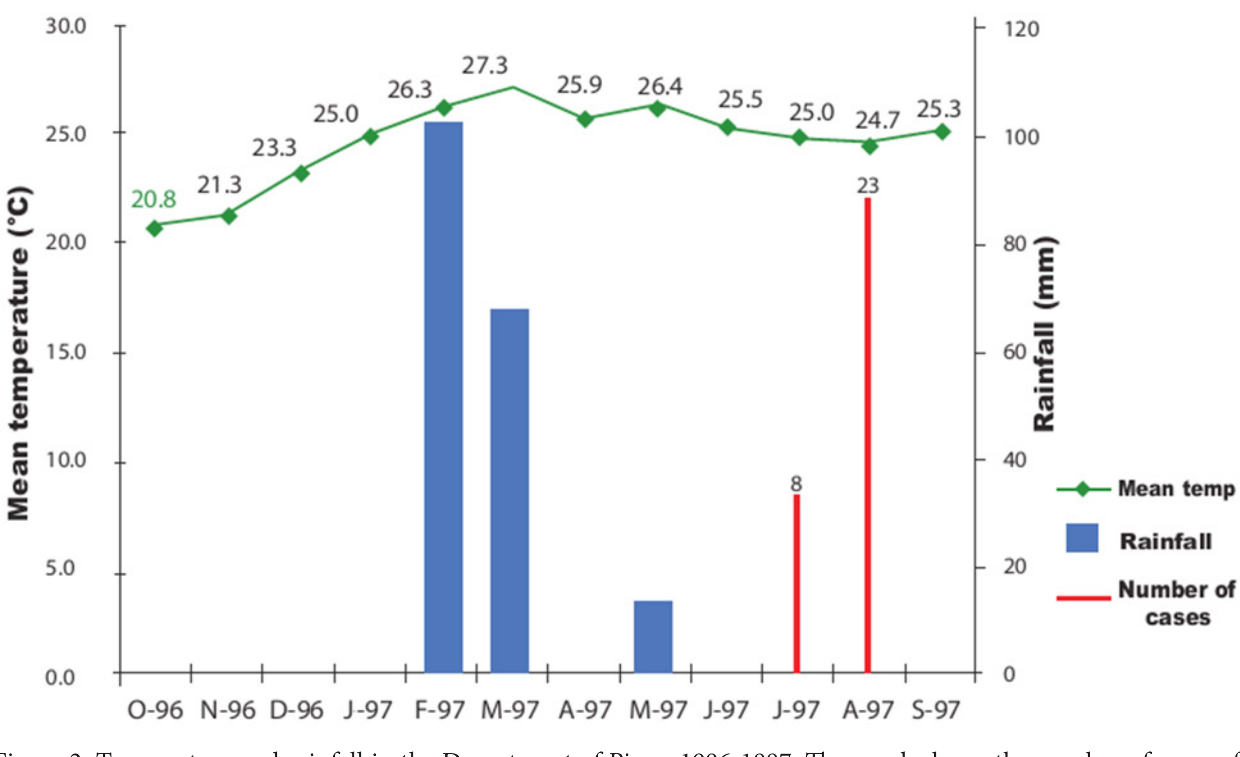

Figure 2. Temperature and rainfall in the Department of Piura, 1996-1997. The graph shows the number of cases of YUOV that occurred after the rainy season. Weather measurements obtained from SENAMHI, Paita Station (Servicio Nacional de Meteorología e Hidrología).

no mosquitoes were found. Other attempts, on 14 and 15 June 2010, to capture mosquitoes were made from 17:00-19:00, from animals in livestock pens behind houses. The procedure was similar to that mentioned above for the Department of San Martín. Note that at all sites, mosquitoes were allowed to feed and were then collected, so that viruses isolated from mosquitoes likely came from the vertebrate hosts.

Isolation of viruses and amplification in cell culture

Pooled mosquitoes were homogenized, clarified, and inoculated onto C6/36 (*Aedes albopictus* mosquito) cells, which were observed for seven days for signs of floating cells (typical effect when C6/36 cells are infected with a dsRNA arbovirus), as previously described (Hurtado-Alendes et al. 2005). Cultures with suspect or obvious cytopathic effects were frozen at -70° C and then passed three more times in C6/36 cells. Usually, suspected positive samples from mosquitoes did not yield clear-cut cytopathic effects even after three passages in C6/36 cells. However, subsequent passages of the majority of these samples in RD (human rhabdosarcoma) cells resulted in clear-cut cytopathic effects. These viruses appeared to replicate slowly in C6/36 cells, a manner different from that of encephalitic alphaviruses but similar to that of other orbiviruses. For many of these orbivirus isolates, it required more than ten days to observe clear-cut cytopathic effects. When the number of infected C6/36 cells increased and formed clusters (30% of cells), the 12 ml culture bottles were shaken to loosen and disperse the clusters and 4 ml of the suspension were transferred to fresh cultures containing half the number of cells normally used. Infected cells in clusters were larger cells than those altered by aging. Passages were frozen when it was clear that 70% of them formed clusters.

Viral RNA was extracted from C6/36 and RD cells by a method standardized for animal samples by separating the detritus by centrifugation at 3,000 rpm x 5 min and then centrifuging the supernatant fluid at 60,000 rpm x 120 min. The pellet was used for extraction of RNA according to Chomczynski and Sacchi (1987) with some modifications. The extracted RNA was analyzed by 1% gel electrophoresis containing 0.5 ug/ml ethidium bromide (Sambrook et al. 1989). Those that showed a suggestive segment profile typical of viruses of the *Reoviridae* family were sent to the Pirbright Institute in Surrey, United Kingdom, for testing by RT-PCR with specific primers (Attoui et al. 2009).

RESULTS

The number of mosquitoes collected in San Martín from 1980 to 1997 is summarized in Table 1. In March of 1997, during the outbreak, we collected mosquitoes of 23 species. Only a few more *Ae. serratus* were collected than in previous years. The most abundant mosquitoes collected were *Ae. scapularis* and *Psorophora cingulata*. Midges of the genus *Culicoides* (family *Ceratopogonidae*) were also present in the collections. In addition, we found mosquitoes of many species that had not been found in previous years in the same locations during the same seasons (Méndez-López and Hurtado-Alendes 2009).

In the department of Piura in 1997, during the outbreak we were not able to capture mosquitoes due to the heavy rain and consequent flooding, which made it difficult to reach those areas. In 1998 we found a lower diversity of mosquitoes in the district of Suyo than in previous years. *Ae. scapularis* was always the predominant species, representing 65.5% (N= 553) of the mosquitoes captured. In June, 2010, we found a high number of *Ae. scapularis* (N=162) in the district of Yamango, the only mosquitoes found in that season (Table 2).

PHSV was isolated from *Ae. serratus* and *Ps. ferox*, sylvatic mosquitoes, and YUOV was isolated from *Ae. scapularis*, a peri-urban mosquito that is widely distributed. Likewise, in Piura, both viruses were recovered from peri-urban mosquitoes; YUOV from *Ae. scapularis* and PHSV from *An. albimanus*, although there were no cases of PHSV in Piura (Table 3).

DISCUSSION

More than 3,500 species of mosquitoes have been recognized world-wide, are distributed throughout temperate and tropical regions of the world, and occupy a wide range of aquatic environments. There are about 1,700 species of culicine mosquitoes distributed in 20 genera, with *Aedes* and *Culex* including the most common arbovirus vectors (Moraes 2007). Tropical humid climates support mosquitoes of the most diverse species, and many of them transmit infectious agents (Brown et al. 2011). Diseases of humans and other vertebrates often are recognized as zoonoses when the habitats of their vectors and reservoir hosts are disturbed, such as after deforestation (Forattini et al. 1995) or dam building (Liehne et al. 1976). Peru, because of its wide variety of habitats, appears to be ideal for mosquitoes of many varieties. Other than studies of yellow fever virus (Mendez et al. 1984), few broad studies have been conducted in Peru, so that relatively little is known about arboviruses in this country.

The Amazon River is the primary source of fresh water for Peru, and the Andes mountains are the main sources of ecosystem variety. As habitat location influences mosquito presence and vectorial capacity to humans and other vertebrates, habitat changes likely affect transmission of potentially emerging diseases and one of the causes of the dispersion of viruses, particularly arboviruses, is the destruction of the natural habitats of their reservoirs or vectors, as has occurred in Peru.

The orbivirus epizootics in San Martin occurred in the first half of 1997 in almost all the provinces of the department associated with the Huallaga River or the Mayo River, causing the deaths of at least 100 horses, as well as some bovids, ovids, and canids. The epidemiological and virological characteristics of the outbreak have been described (Attoui et al. 2009). PHSV affected only horses but YUOV affected cattle and donkeys. Cattle and dogs from the neighboring Department of Ucayali also were affected.

In the basin of the Mayo River the peak number of cases of PHSV during the outbreak in the rainy season coincided with the highest humidity but not with the highest temperature,

Table 1. Number of arthropods collected in the upper jungle of the Department of San Martín, 1980-1997 by species and month-year of collection.

	MONTH AND YEAR OF COLLECTION				
SPECIES	Mar-1980	Mar-1982	Mar-1983	Mar-1984	Mar-1997
Psorophora ciliata	6	-	56	-	-
Psorophora cingulata	-	-	-	-	120
Psorophora albigenu	-	-	-	-	12
Psorophora linneata	25	-	-	1	-
Psorophora albipes	267	-	-	-	-
Psorophora ferox	10	160	-	-	115
Psorophora cilipes	181	-	-	-	-
Psorophora lutzi	-	77	-	33	110
Psorophora lannei	-	-	-	17	-
Haemagogus janthinomys	2	20	5	2	4
Aedes aegypti	-	-	-	-	10
Aedes fulvus	141	73	-	-	50
Aedes upatensis	-	5	-	-	-
Aedes pennai	-	1,259	-	24	-
Aedes species	-	1	-	-	150
Aedes scapularis	179	204	-	15	180
Aedes serratus	7	7	-	1	90
Aedes species	-	-	-	-	123
Limatus paraensis	-	3	-	-	15
Limatus durhamii	-	5	-	14	25
Trichoprosopon brevipes	-	-	20	-	-
Sabethes belisarioi	2	1	6	3	-
Sabethes species	-	-	1	-	50
Mansonia pseudotitillans	-	-	6	-	-
Mansonia venezuelensis	-	-	10	-	-
Mansonia indubitans	-	-	-	-	30
Wyeomyia moerbista	-	-	-	1	-
Anopheles rangeli	-	-	-	-	90
Coquillettidia venezuelensis	-	-	-	-	12
Culex insinuatus	-	-	-	-	10
Culex paraensis	-	-	-	-	6
Culex (Melanoconion) species	-	-	-	-	50
Culex species	-	-	-	-	75
Culicoides species	-	-	-	-	160
Simulium species	-	-	-	-	70
Total species	10	12	07	10	23
Total mosquitoes	820	1,815	104	111	1,557

Table 2. Mosquitoes collected on the high side of the Piura River: Ayabaca (Suyo) and Morropón (Yamango), provinces, Peru, 1981-2010, by species and month-year of collection.

SPECIES	LOCATION						
	Suyo (Mar-1981)	Suyo (Feb-1983)	Suyo (Jun-1996)	Suyo (Aug-1996)	Suyo (Jan-1998)	Suyo (Mar-2008)	Yamango (Jun-2010)
Anopheles calderoni	923	81	72	37	0	6	0
An. albimanus	454	1,531	581	30	65	1,140	0
An. pseudopunctipennis	12	0	0	18	0	70	0
Aedes scapularis	230	77	1,180	61	6	186	162
Ae. taeniorhynchus	0	34	154	107	0	0	0
Culex quinquefasciatus	40	16	154	107	7	0	0
Culex species	0	0	47	5	0	43	0
Culex (*Melanoconion*) species	6	0	0	43	0	101	0

which occurs in October. On the other hand, isolated cases of YUOV were found between April and August, during the season called dry or cold, when temperature and humidity do not vary but rains decrease (Figure 3).

It is likely that the epidemiology of PHSV follows ecological patterns associated with the characteristics of the vectors, similar to those of other arboviruses (Soldán and Gonzales-Scarano 2005). The PHSV outbreak in 1997 peaked between January and May, with about half the cases occurring in March, coincident with the highest humidity of the year (85.5%). *Ps. ferox* and *Ae. serratus* have historically been collected in natural woodlots throughout the Americas (Forattini et al. 1968). The biological cycles of these mosquitoes are highly dependent on temperature, relative humidity, and rainfall (Loetti et al. 2007). However, in the areas affected by the PHSV outbreak, it was uncommon to find them feeding on horses in large numbers until 1997. During previous entomological investigations in the same departments, we observed that these mosquitoes were also present (Mendez-Lopez, unpublished observations), but we did not isolate PHSV from them nor did we find mosquitoes of a wide variety of species, even during March (Table 3). It is likely that other factors, such as mosquito overwintering strategies that favor an increase in the adult population of orbivirus vectors, can ensure the interrelation with the host and the reservoir for subsequent epizootic outbreaks. In 1996, in the Province of Rioja, the lowest levels of precipitation in the previous 30 years occurred. This situation could have created an environment in which the eggs of some mosquitoes resisted desiccation and eclosed when the precipitation increased in 1997 (Crans 2004, Clements 1992). Although many variables can affect mosquito population dynamics, one reasonable hypothesis for the increase of mosquito species in the area is the increased level of precipitation in October, 1996 along the tributary rivers of Mayo River, causing considerable flooding for a month afterwards. These conditions, along with high temperatures, created optimal conditions for oviposition by a variety of mosquito vectors.

Yunnan virus is transmitted by *Ae. scapularis*, a mosquito dependent on variable temperature, relative humidity, and

Table 3. Species of mosquitoes infected with Peruvian horse sickness virus (PHSV) or Yunnan virus (YUOV) during a 1997 outbreak, and from 1998 and 2010 samplings in the Departments of San Martín and Piura. The 13 orbivirus strains were isolated from mosquitoes. Only YUOV was recovered from *Ae. scapularis* on the coast and in the jungle. PHSV was recovered from sylvatic mosquitoes in the jungle and from *An. albimanus* on the coast.

Species (No. of pools)	Department	Date	Province	Locality	Virus
Ae. serratus (1)	San Martín	March 1997	Moyobamba	Jepelacio	PHSV
Ps. ferox (2)	San Martín	March 1997	Moyobamba	Habana	PHSV
Ae. scapularis (2)	San Martín	March 1997	Moyobamba	Jepelacio	YUOV
Ae. scapularis (2)	San Martín	March 1997	Mariscal Cáceres	Juanjui	YUOV
Ae. scapularis (1)	Piura	Jan 1998	Ayabaca	Suyo	YUOV
An. albimanus(3)	Piura	March 2008	Ayabaca	Suyo	PHSV
Ae. scapularis (2)	Piura	June 2010	Morropón	Yamango	YUOV

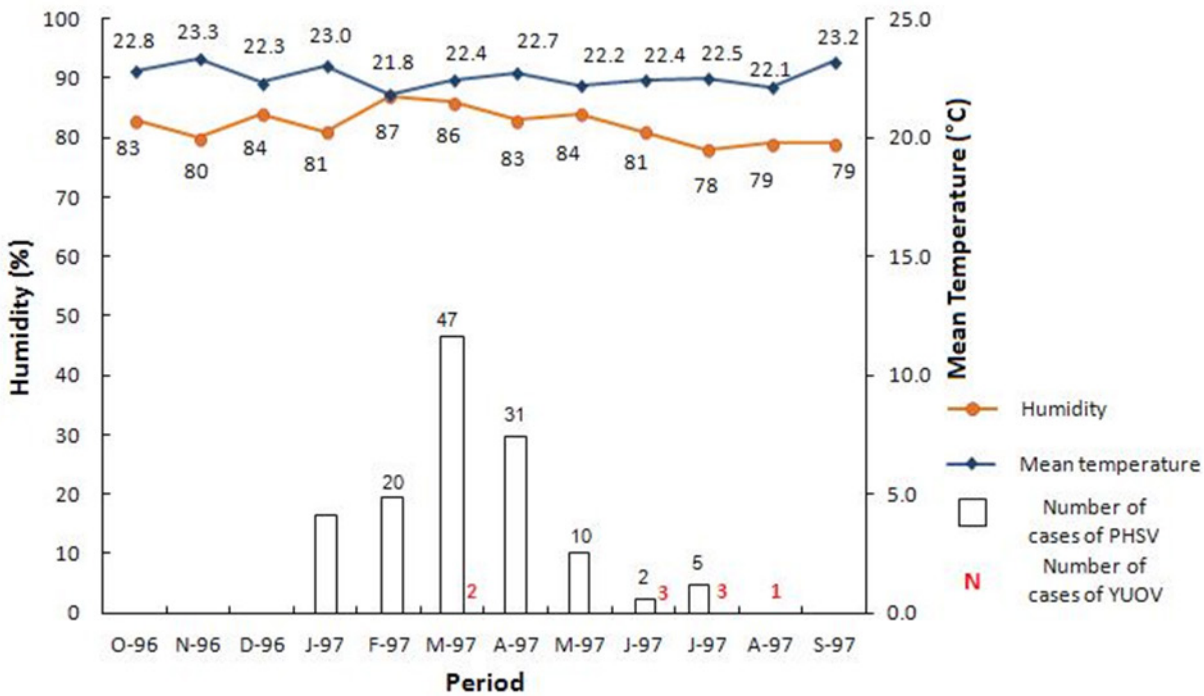

Figure 3. Temperature and humidity in the Department of San Martín, 1997. The graph shows the number of cases during the outbreak of Peruvian horse sickness virus (PHSV) and Yunnan virus (YUOV). Weather measurements obtained from SENAMHI, Moyobamba Station (Servicio Nacional de Meteorología e Hidrología, Moyobamba, Peru).

rainfall associated with the ecology of certain locations, vegetation coverage, and modified environments (Guimaraes et al. 2000, Forattini and Gomes 1988) for population increases, with wide distribution in secondary vegetation and peridomiciliary habitats (Diéguez et al. 2005, Méndez et al. 2001). This mosquito is present in the diverse environments of Peru and in the continent as a whole (Méndez et al. 2001, Reinert et al. 2005). At the end of March, 1997, the intensity of the El Niño began to increase, with temperatures increasing throughout the year and even into April, 1998 (Galarza and Kámiche 2012). In this study, *Ae. scapularis* were captured from bovids both during the day and, particularly, in the evening, and from other domestic animals (Forattini and Gomes 1988).

Capture of mosquitoes in the Piura River basin has been a routine activity done by our laboratory during surveillance for Venezuelan equine encephalitis virus when the weather creates transient flooding of rice paddies. In January, 1998, the high side of the river was still experiencing heavy rain due to the El Niño, and we were unable to capture many mosquitoes and did not isolate virus from the few we did capture. In March, 2008, in a resting place of bovids in Suyo District, we found high densities of *An. albimanus* and *Ae. scapularis*. That year we recovered PHSV for the first time from *An. albimanus* and YUOV from *Ae. scapularis* in that location. Subsequently in Yamango, in June, 2010 (dry season), we recovered YUOV from *Ae. scapularis* (Table 2). However, after the epizootic of 1997, YUOV was isolated sporadically from mosquitoes and bovids on the high side of the Piura River. It is likely that in these places the virus was enzootic.

On the low side of the river, during the rainy seasons and when the highest temperature (34° C in March) was observed, we captured *Ae. scapularis*, mostly in the salt water areas of the coast. The captures were made mainly using biting collections from burros, and these mosquitoes were even in greater numbers than in the jungle of San Martín. No arboviruses were isolated in those years (Table 3).

It is possible that the transmission of Middle Point and Yunnan viruses in Australia, China, and Peru involves vectors with similar bionomic requirements for their circulation in widely disparate geographic areas, all of them located in the intertropic zone. Arthropods depend on local levels of temperature, humidity, and precipitation for reproduction and on overall ecological aspects for their maintenance and distribution (Lord 2004); these areas may have more similarities than are currently recognized.

Another possible variable for the emergence of PHSV and YUOV may be human activities. Deforestation that generates extensive beaches in the hydrography of the Huallaga River and the Mayo River and their tributaries during the rainy season, are appropriate for oviposition by female mosquitoes of various species, including vectors of arboviruses identified in similar ecological zones (Aguilar et al. 2007). Mosquitoes of four species of the genus *Psorophora* are likely to be favored under such conditions (Table 2). Excessive rains cause overflows in the river basins, leading to the development of mosquitoes of this genus in the bends of the Huallaga River. *Ae. serratus* and *Ps. ferox* living in the forest environment have little possibility of moving to open land and they probably infect resting horses during the day.

It is known that the Amazon Basin has the highest prevalence of arboviruses in the world, because their natural cycles involve jungle mosquitoes and vertebrate reservoirs, including birds and rodents (Turell et al. 2005, Ferro et al. 2003). Based on our surveillance program, *Ae. scapularis* appears to be the primary vector of YUOV in this area. These mosquitoes are also present in contiguous saline areas on the low side of Piura River, but no cases of undiagnosed encephalitis have been reported there. Considering the wide distribution of these mosquitoes in South America, YUOV may already be present in countries bordering Peru.

Until now, it has not been possible to determine the role of *An. albimanus* in the transmission of PHSV, since there have been no reports of sick horses in the area where PHSV was recovered from these mosquitoes. In January, 1996, during surveillance for arboviruses, it was found that some pools of *An. albimanus* were infected with a then-unidentified virus, with rice cultivation being the major contributor to the high density of this vector at the collection site. Subsequent studies showed that the virus was PHSV. Additional studies are needed in order to understand the economic impact of these orbiviruses, to determine the seasonal conditions leading to amplification of these viruses, and to understand the complexities of the eco-epidemiologic situation with regard to the interrelations of host, vector, and virus.

Acknowledgments

We thank Mr. Roberto Fernandez, entomologist NAMRU-6, Lima, and Mr. Walter León, entomologist from National Institute of Health, Lima, for providing the taxonomic placement of the mosquitoes we collected. This study was funded by The Faculty of Medicine of San Martín de Porres University (USMP) Lima-Peru, project E100012010042.

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